METAL BINDING PROTEINS, RECOMBINANT HOST CELLS AND METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of United States Provisional Application No. 60/240,465, filed October 12, 2000, which is incorporated herein to the extent that there is no inconsistency with the present disclosure.

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BACKGROUND OF THE INVENTION

The field of the present invention is the area of molecular biology, in particular as related to a metal-binding protein produced through recombinant DNA technology.

One of the best-characterized mercury resistance (*mer*) operons is located on transposon Tn*21* from the *Shigella flexneri* IncFII plasmid R100. This operon consists of five structural genes - *merT, merP, merC, merA,* and *merD* - and a regulatory gene, *merR* (Fig. 1). MerT, MerP, and MerC are involved in the transport of Hg(II) into the cell. MerA is a cytosolic, NADPH-dependent, flavin adenine dinucleotide-containing oxidoreductase which reduces Hg(II) to Hg(0). The *merR* gene is transcribed in the direction opposite from that of the structural genes. Its product, MerR, represses expression of the *merTPCAD* genes in the absence of Hg(II), activates their expression in the presence of Hg(II), and represses its own

expression in the presence or absence of Hg(II). MerD plays a minor role in regulation, possibly as an antagonist of MerR, which reestablishes repression of *merTPCAD* once Hg(II) has been reduced to Hg(0).

Extensive genetic and biochemical data indicate that the protein contains three domains [Ross et al. (1989) *J. Bacteriol.* **171**:4009-4018; Shewchuk et al. (1989) *Biochemistry* **28**:2340-2344; Summers, A.O. (1992) *J. Bacteriol.* **174**:3097-3101]: a helix-turn-helix DNA-binding domain from L10 to R29 [Brennan et al.(1989) *J. Biol. Chem.* **264**:1903-1906; Livrelli et al. (1993) *J. Biol. Chem.* **268**:2623-2631]; a "coupling" domain from K30 to H81 which may convey the status of the Hg(II) binding site to the DNA binding site [Comess et al. (1994) *Biochemistry* **33**:4175-4186; Helmann et al. (1990) *Science* **247**:946-948]; and a long helical region from C82 to C117 which constitutes both the dimer interface and, with the loop containing C126, the Hg(II)-binding domain [Zeng et al. (1998) *Biochemistry* **37**:15885-15895].

The MerR homodimer binds Hg(II) by using the thiols of three conserved cysteines: cysteine 82 (C82) from one monomer and cysteines 117 and 126 (C117 and C126) from the other monomer. These ligands form a novel planar tricoordinate complex with Hg(II) [Helman et al. (1990) supra; O'Halloran, T.V. (1993) *Science* **261**:715-725; Utschig et al. (1995) *Science* **26**:380-385; Wright et al. (1990) *J. Am. Chem. Soc.* **112**:2434-2435]. Upon binding Hg(II), MerR undergoes a conformational change that leads to an underwinding of the P_r region and thereby enables RNA polymerase to form an open complex. Curiously, although the MerR homodimer contains two potential Hg(II) binding sites, the purified protein binds only one Hg(II) per dimer. Moreover, although the two other group 12 metals, Zn(II) and Cd(II), also form stable complexes with protein thiols [Boulanger et al. (1983) *Proc. Natl. Acad. Sci. USA* **80**:1501-1505; Furey et al. (1986) *Science* **231**:704-708; Santos et al. (1991) *J. Am. Chem. Soc.* **113**:469-474; Utschig et al. (1993) *Methods Enzymol.* **226**:71-97], purified MerR binds Hg(II) preferentially even in the presence of a 1,000-fold excess of Cd(II) or Zn(II) [Shewchuk et al. (1989) *Biochemistry* **28**:2331-2339] and also requires 100- to 1,000-fold higher concentrations of these metals for transcriptional activation [Ralston et al. (1990) *Proc. Natl. Acad. Sci. USA* **87**:3846-3850.

Although several studies have described mutants altered in either repression or activation, no genetic study of *merR* has explicitly addressed the basis of its metal specificity prior to Caguiat et al. (1999) *J. Bacteriol.* **181**:3462-3471. Caguiat et al. report variants of MerR with an altered response to a metal; the properties and locations of these mutations shed light on the basis for metal-provoked activation by MerR. That also led those authors to examine the possible occurrence of similar secondary-structure elements in other members of the MerR family, and our findings in the latter regard indicate conservation of a structural domain in a subset of this family.

There is a long felt need in the art for compositions and methods useful in the remediation of materials contaminated with heavy metal ions, especially mercury and cadmium. The present invention provides artificial metal binding proteins, coding sequences, recombinant DNA molecules, recombinant host cells and methods for production of the artificial metal binding proteins.

SUMMARY OF THE INVENTION

The present invention provides DNA sequences encoding artificial metal binding proteins, which are termed chelons herein, and DNA sequences encoding the mercury binding protein MerR (derived from Tn21), vectors comprising those sequences operably linked to promoters functional in particular host cells of choice, recombinant host cells expressing the chelon proteins or MerR protein and methods of producing the chelon proteins and MerR protein recombinantly. Host cells include, but are not limited to, bacterial cells (e.g., *Escherichia coli*, *Bacillus subtilis*, Pseudomonas species, etc.), yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris*), fungi (e.g., Aspergillus species, *Trichoderma reesei*), plant cells (e.g., Arabidopsis, tobacco, petunia, Populus species, Salix species, among others) and animal cells such as CHO cells or avian cells.

The present invention further provides chelon proteins which bind heavy metal ions, including but not limited to divalent cadmium and mercury ions, with relatively high affinity. The amino acid sequence of a mercury-specific chelon is disclosed in Table 1D, and the nucleotide sequence encoding it is present in Table 1C. Additional chelons which bind both